Posters: Diffraction and Scattering Techniques

2648-Pos
Bayesian structure determination from fluctuation X-ray scattering experiments
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Fluctuation X-ray scattering is an emerging method for biomolecular structure determination, where scattering data of an ensemble of molecules in a dilute solution is collected using ultra-short X-ray pulses below rotational tumbling times. This allows to capture structural information in angular intensity correlations that are absent in traditional solution X-ray scattering methods, while also keeping the more biological conditions in solution compared to single-molecule X-ray scattering. However, the reconstruction of the molecular structure of the sample using the scattering images poses a significant challenge, since the orientations of the individual molecules in the solution are unknown and the low signal-to-noise ratio has to be overcome. We present a rigorous Bayesian framework that finds the molecular structure that has the largest probability given all recorded scattering images. In this approach, the orientation of each molecule explicitly appears in the likelihood function. We formally integrate out the unknown individual orientations, and to reduce the computational cost, this integration is approximated by a finite sum over randomly chosen orientations of each molecule. We show that our method can recover the molecular structure of a fictitious 12-atom molecule up to 4-Å resolution, using 2000 synthetic noise-free images of 50 randomly oriented copies each.

2649-Pos
Using macromolecular phasing and refinement techniques for a small molecule gyrase inhibitor
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Topoisomerases resolve DNA topoisomers, which is essential for faithful replication and transcription. Gyrases are type II topoisomerases unique to bacteria and well-known targets for antibiotics. Unfortunately, currently used gyrase inhibitors suffer from increased resistance, necessitating structure-based drug design on novel chemical classes. During such efforts, a crystal of a small molecule gyrase inhibitor grew fortuitously from an experiment aimed at a protein/ligand complex. The crystal diffracted insufficiently for standard direct phasing methods to work, and the diffraction data to 1.3 Å resolution did not contain anomalous signal for experimental phasing. Using macromolecular replacement techniques with fragments of the ligand as search models that were predicted to be planar, an initial electron density map was obtained that allowed building of a complete structure. While standard small molecule refinement was unstable, macromolecular refinement protocols were applied successfully to an R_{free} of ~20%, likely aided by the appreciably large solvent content of the small molecule crystal (34-51%, depending on how it is calculated). Comparison of the small molecule structure to a chemically related ligand in complex with the ATPase domain of *P. aeruginosa* gyrase reveals quite different conformations due to variations in torsion angles connecting aromatic and aliphatic moieties. Such information may prove useful in drug design. While the present case may be among the first in using ''macromolecular'' methods for phasing and refinement of ''small-molecule data,'' we hope that in the future this approach proves useful in extracting information from sub-standard small-molecule diffraction data.

2650-Pos
Femtosecond imaging of giant-hemeprotein with XFEL pulses
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Outrunning radiation damage, extremely intense femtosecond pulses of x-ray free-electron lasers (XFELs) open up the possibility of imaging the structure and dynamics of macromolecules frozen in time at room temperature. Single-Particle Imaging (SPI) of uncrystallized macromolecules at high-resolution is one of the most-desired foundational applications of XFELs and one of the ultimate goals of SPI is to image the light-induced ultrafast (ps/fs) dynamics in single-macromolecules. Biological proteins exhibiting photoinduced ultrafast dynamics are in the range of tens to few-hundreds of kDa, which are too small for SPI with the photon flux of current generation XFELs. Ideally, one needs MDa-sized, strongly scattering, photoactive proteins for such challenging endeavours to discern the signal above the background scattering and to obtain difference-maps with sufficient quality to elucidate photoinduced changes empirically. Photoactive proteins are sparse in nature and MDa-sized photoactive macromolecules are extremely rare. We have found one such rare MDa-sized photoactive, porphyrin-containing, large-metalloprotein-complex, the giant-hemeprotein—erythrocruorin (Ery) and show that Ery is likely suitable for validating the ultimate potential of SPI. We present the cryoEM characterization of stability of Ery in the unique experimental conditions of XFEL-SPI together with simulated single-molecule diffraction and 3D intensity reconstruction using expand-maximize-compress (EMC) algorithm and propose a potential SPI roadmap to demonstrate the imaging of ps/fs resolved bio-functional dynamics in Ery with XFEL pulses. Also, here we report the first in-solution ensemble fluctuation scattering results of Ery with the microfocus hard x-ray FEL pulses at the Linac Coherent Light Source (LCLS), USA—a step towards single-molecule imaging of Ery in-solution with nanofocus. We envisage that the robust giant-hemeprotein Ery could likely be an archetype biological macromolecular system enabling the imaging of light-induced ultrafast (ps/fs) dynamics such as protein-quake following heme-doming in isolated single-proteins—''the Holy Grail'' of XFEL-SPI.

Posters: Optical Spectroscopy: CD, UV-VIS, Vibrational, Fluorescence

2651-Pos
Fluctuation of Raman scattering can be an initial marker for iPSC differentiation
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Change in the intensity of Raman scattering peaks from living cells or tissues have been widely used to estimate the type or state of the specimen. Transition of cell state can also be estimated by Raman spectrum of the cells; however, subtle change such as initiation of differentiation is difficult to detect because of the low signal of the Raman scattering. In this study, we have successfully detected the onset of the iPSC differentiation by using the fluctuation of the Raman scattering peak intensities during cardiomyogenesis. To detect the fluctuation of the Raman peaks in non-labeling and non-destructive manner, we have developed a technique to collect Raman scattering spectra with high reproducibility, independent of experimental date, analyst, and optical aberrations. Using this newly developed method, small fluctuation in Raman peaks can be observed. Fluctuation in Raman peaks was observed earlier than the change in Raman peak intensities, showing that fluctuation-based analysis can be useful in detecting the onset of cell state transition. We also applied Dynamic Network Biomarker (DNB) based analysis to the obtained dataset, which also showed earlier detection of the onset of cell state transition, indicating that fluctuation-based analysis will be a strong tool in cell spectrum analysis.

2652-Pos
Development of a confocal system for bioimaging
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The goal of this work is to build a confocal microscope for imaging biological tissues. The purpose for building a confocal system from scratch is to enable flexibility of source and detection as well as future implementation of multimodal imaging methods such as optical coherence tomography. The system is being designed and built by undergraduate students in physics and engineering at